REGULATION OF PATTERN OF GENE EXPRESSION BY CONTROLLING ELEMENTS IN MAIZE

Barbara McClintock

The production of anthocyanin pigment in maize requires the functioning of a number of known genes distributed among the chromosomes of the complement.. This pigment may be produced in most parts of the plant. Its distribution and intensity vary widely in different strains of maize. Each pattern reflects the action during development of genetic mechanisms that regulate pigment production. One of the gene loci participating in anthocyanin formation is the C_2 locus in chromosome 4. Two independent events brought the action of the gene at this locus under the control of the *Spm* (Suppressor-mutator) system. The symbols $c_2{}^{m-1}$ and $c_2{}^{m-2}$ designate the modified loci resulting from these events. In both instances, the element of the *Spm* system that is inserted at the C_2 locus contributes to regulation of pattern of anthocyanin production in plant and kernel. It can induce different patterns. A change from one pattern to another may be traced to modifications of the gene locus initially produced by a response of the element to an active *Spm* element. Some of these responses may occur in individual cells early in development. The locus is thereby "set" at an early stage to induce a particular pattern of anthocyanin distribution in sporophytic tissues formed by descendants of such a cell. Some of the settings may subsequently be "erased," probably in those progeny cells that are included in the germ line. The evidence for such settings and erasures is the subject of this report.

The C_2 gene locus was selected for these studies because modifications of control of its action may be detected in all parts of the plant: root, stalk (including interior cells), leaf parts, tassel parts (including the anthers), husks, and portions of the cob, including interior cells as well as floral parts. Alterations of control of C_2 action may also be detected in the pericarp layer of the kernel, which is derived from maternal cells, and in the aleurone layer of the endosperm.

The studies to be reported required the use of a recessive allele of C_2, initially isolated and examined by E. H. Coe. It will be referred to as the standard recessive and symbolized as c_2. When a plant is homozygous for c_2, anthocyanin pigment is produced in many parts of the plant but its intensity is low. The aleurone layer of kernels homozygous for c_2 is devoid of the pigment. When the standard C_2 locus is present in a plant or kernel, on the contrary, pigmentation is intense, provided all other genes involved in anthocyanin production are active within the tissues.

The most revealing studies of altered programming of action of the C_2 locus were conducted with plants having a genic constitution that would have allowed intense pigment to be produced in the various parts of the plant had the standard C_2 gene been present rather than c_2^{m-1} or c_2^{m-2}. These plants were either c_2^{m-1}/c_2 or c_2^{m-2}/c_2 in constitution, and each had one or more *Spm* elements whose components were fully active, at least initially, in the young plant. The state of c_2^{m-1} that was available for these tests is one that responds to *Spm* late in development, whereas one of the states of c_2^{m-2} can respond very early. Although the types of modification produced by these responses to bring about altered pigment distributions and intensities were similar in the two instances, those occurring at c_2^{m-2} were more instructive, for the following reason. Early-occurring alterations that modify the programming of anthocyanin production are made evident in the plant in large sectors, which include within them various different tissues. Distribution of anthocyanin to these tissues, or its intensity in any one tissue or tissue part, may be compared with that in other sectors in the same plant.

Most of the examined plants produced tillers (side branches) with fertile ears. Observations were made of anthocyanin pigment distribution in the tissues of the main stalk and in those of the tillers. The ears of each plant were pollinated by plants that were homozygous for the standard recessive c_2 (whose action is not altered by *Spm*) and also for wx^{m-8}; these plants had no active *Spm* element. *Wx* produces amylose starch in pollen grain and endosperm; *wx*, the standard recessive allele, does not produce this starch. When wx^{m-8} is present, the action of the gene at this locus is under the control of the *Spm* system. Since the ear-bearing c_2^{m-1}/c_2 and c_2^{m-2}/c_2 plants were also either *Wx/wx* or *wx/wx* in constitution, the incorporation of wx^{m-8} in the testcross allowed a determination of the presence or absence of *Spm* in the cells producing each ear, and also the state of *Spm* if present. Ears are especially useful for study of patterns of anthocyanin pigment distribution, because the pigment is preserved in the cells of the dried ears, and its distribution in parts of the cob as well as in the pericarp and aleurone layers of the kernel can be examined. Many of the ears had sectors of various sizes that exhibited altered patterns of distribution of pigment in the cob and kernels. Each sector arose from the progeny of a single cell in which a modification had occurred at the c_2^{m-1} or the c_2^{m-2} locus. Comparison of types of pigment distribution in the various tissues within the sectors on a single ear, or on different ears of the same plant, gave evidence of the range of altered programming that can be induced by responses of the controlling element to *Spm*. When intensity as well as distribution of pigment was considered, the range proved to be wide.

The tests were conducted with several hundred plants carrying one state of c_2^{m-1}, and with several hundred carrying one or the other of two selected states of c_2^{m-2}. Because the results obtained with the differently constituted plants are comparable, discussion will be confined to those obtained with one state of c_2^{m-2}.

Pigment Distribution in Parts of the Ear

A few illustrations will facilitate discussion. Two ears produced on the main stalk of one plant (8597C-4) that was c_2^{m-2}/c_2 in constitution are shown in Plate 1*A*. The silks of these ears received pollen from plants that were homozygous for the standard recessive c_2, and for wx^{m-8}, and had no active *Spm*. Thus at least half the kernels on each ear should have been homozygous for c_2 and should have had no pigment in the aleurone layer. The ear in the lower part of the photograph had this phenotype. On that ear two additional classes of kernels appeared, one showing spots of pigment in a colorless background, the other intensely and uniformly pigmented. The spotted kernels received a c_2^{m-2} locus that responded to *Spm* in

development of the endosperm in the manner expected of an unmodified c_2^{m-2} locus. The fully pigmented kernels received a c_2^{m-2} locus that had been modified so that the gene functioned in the cells of the aleurone layer in a manner resembling that of the standard C_2 gene. At the tip of the ear there was a large sector in which all the kernels that received a derivative of c_2^{m-2} expressed this phenotype. In these kernels the pericarp layer also was densely pigmented, except at the region of the crown. A discussion of pericarp pigmentation patterns will be deferred until later; here it need only be stated that not all the kernels on this ear had pigment in the pericarp. Some had none; in others it was confined to well-defined sectors within the layer. A part of the ear shank, visible in the photograph, was variegated for anthocyanin pigment, as were the parts of the cob.

All kernels on the other ear produced by the same plant were colorless. No pigment appeared in the aleurone layer or the pericarp layer, and the cob parts showed no evidence of variegated patterns of pigmentation. The cells that gave rise to this ear had more than one active *Spm* element. Thus the uniformity of gene expression throughout the ear may not be ascribed to somatic loss of *Spm* or of its activity. A specific type of modification at the locus of c_2^{m-2} must have occurred in the cell whose descendants produced this ear.

Plate 1*B* shows the cobs of three ears produced on another plant (8500-10)—the two on the right by the main stalk, the one on the left by the tiller. An active *Spm* element was present in the cells that gave rise to all these ears. The deep pigment in the floral parts, visible as distinct sectors in the two cobs produced by the main stalk, resembled that which would have appeared throughout the cob had the standard C_2 allele been present. In addition, the pericarp layer of the kernels located within these sectors was deeply pigmented. The aleurone layer of all kernels on the right-hand cob was totally colorless, indicating that the c_2^{m-2} locus had been modified in the cell whose descendants produced the ear. No early modification occurred in the cells giving rise to the middle ear, not even in the one whose descendants produced the dark-pigmented basal sector in the cob. On the left-hand ear all kernels that received a derivative of c_2^{m-2} had deep and uniform pigmentation in the aleurone and pericarp layers. Thus the early modification of c_2^{m-2} in the cell whose descendants produced the ear induced a new pattern of anthocyanin distribution in the plant parts, allowing a C_2 type of expression in the maternally derived pericarp layer and in the aleurone layer of the endosperm but not in the floral parts of the cob.

Cobs of four ears produced by another plant (8595B-1) are shown in Plate 1*C*. The cob at far right, from the ear of the main stalk, had many small sectors showing pigmentation of different intensities in the floral parts. The other three cobs came from ears produced by the three tillers of the plant. The one pictured second from right had no deeply pigmented sectors. The adjacent cob on the left was uniformly and deeply pigmented in all its parts. The leftmost cob had both large and small sectors with intense pigment in the floral parts. Kernels on the two right-hand cobs had pigmentation patterns in the aleurone layer indicating that no early modification of the c_2^{m-2} locus had occurred to alter gene action in that layer. Such a modification did occur during development of each of the two tillers whose cobs are shown on the left. The event in the cell whose descendants produced the leftmost cob did not allow pigment to be formed in the aleurone layer of its kernels. In the tiller whose cob is adjacent, a very early-occurring event at c_2^{m-2} caused the gene to act like the standard C_2. The altered expression was evident in all parts of the tiller and also in the aleurone layer of kernels on its ear. All those that received the modified c_2^{m-2} locus had deep, uniform pigmentation in that layer.

The cobs and parts of the husks of two

ears from plant 8595C-9 are shown in Plate 1*D*. The cob on the right was produced by the main stalk and the one on the left by the tiller. In the right-hand cob, pigment in the husks and floral parts resembled that appearing when the standard C_2 allele is present. The pericarp layer of the kernels on this ear was also deeply pigmented except at the very tip of the ear, where only sectors of deep pigment in a colorless background appeared. The modification of c_2^{m-2} that induced C_2 type of expression in the husks and cob did not do so in the aleurone layer of the kernels. Kernels that received both c_2^{m-2} and *Spm* had spots of pigment in a colorless background in the aleurone layer, as did kernels of the same constitution on the tiller ear.

Plate 1*E* shows cobs of two ears of plant 8595B-5. The right-hand cob, produced by the main stalk, had many small sectors with pigment of various intensities. The one on the left, produced by the tiller, had one large sector with deep pigment in the floral parts and several smaller sectors of this type. Control of aleurone pigmentation pattern was not altered in kernels of the right-hand cob. It was altered in kernels of the left-hand cob: all those that received the modified c_2^{m-2} locus had deep pigment, uniformly distributed throughout the aleurone layer. Though the pericarp layer of all kernels on this ear was darkly pigmented, many kernels exhibited sectors of even more intense pigmentation in this layer. An active *Spm* was present in the cells that gave rise to both of these ears.

Plate 1*F* shows the cobs of two ears of plant 8595B-7. The one on the right was produced by the main stalk and that on the left by the tiller. In the cells that gave rise to these ears, no change had occurred to alter the action of the gene in the aleurone layer of the kernels, not even in that part of the tiller ear where the floral parts were deeply pigmented. All the kernels within that sector, however, had deep pigment in the pericarp layer.

The examples in Plate 1 give evidence of altered patterns of distribution of anthocyanin pigment, each referable to a modification of the c_2^{m-2} locus in an individual cell during plant development as a result of a response of its controlling element to *Spm*. Some modifications effected a pattern resembling that produced by the standard C_2 allele. Others, in combination with the standard recessive c_2, present in all cells of the examined tissues, induced patterns resembling the one that appears when this recessive is homozygous. Many modifications, however, gave rise to pigment distributions and intensities among the different tissues that did not conform to either of these phenotypes, as indicated in Table 2.

TABLE 2. Combinations of Phenotypic Expression That May Appear in Cob and Kernels as the Result of Different "Settings" of the c_2^{m-2} Locus*

In Floral Parts of Cob	In Layers of Kernels	
	Pericarp Layer	Aleurone Layer
C_2	C_2	C_2
C_2	C_2	c_2
c_2	C_2	C_2
c_2	c_2	c_2
C_2	C_2	unchanged
C_2	unchanged	unchanged
unchanged	C_2	C_2
c_2	unchanged	unchanged
c_2	c_2	unchanged
c_2	C_2	c_2

* C_2: phenotype resembling that produced by the standard C_2 locus. c_2: phenotype resembling that produced by the standard recessive c_2. Unchanged: c_2^{m-2} locus unmodified in its expression.

Pigment Distribution in the Pericarp Layer of the Kernel

The illustrations of altered patterns of pigment distribution in kernels and cobs (Plate 1) are supplemented by several others that show pigment distributions in the pericarp layer of the kernel. The pericarp is the outermost tissue layer of the kernel. It represents the ovary wall and thus the cells composing it are maternal

in origin. When pigment appeared in this layer in *mature* kernels on ears of the investigated plants, its intensity was often low, particularly in the region of the crown (Plates 1*A* and 2*D*). In contrast, the pigment might be intense in all parts of the pericarp layer in kernels that commenced development after pollination but, for some yet undetermined reason, ceased development early. Many kernels of this type appeared on ears that had been pollinated on the same morning in July 1965. This was fortunate, for those kernels provided an opportunity to examine and compare the many different patterns of pigment distribution and intensity occurring in the pericarp layer. Such kernels are small and hollow when dry, as they contain neither embryo nor endosperm tissue. (See the hole in the second kernel from right, lower row, Plate 2*A*.) They will be referred to as abortive kernels.

The ovary wall is initiated by a ring of cells about the base of a growing point that will form the nucellus tissue in which the megasporocyte will be differentiated. The ring grows upward and over this nucellus initial, gradually enclosing it by a narrowing of the ring and a final coming together of its parts at the position of the future attachment of the silk. The silk represents an extension of growth of one section of the ring. This manner of development of the ovary wall is well illustrated by the pigmentation patterns of those abortive kernels in Plate 2*A*,*B* that have deep-pigmented sectors in a colorless background. The large pigmented sectors are continuous and extend from the base of the kernel to the point of silk attachment. The silk, broken near its attachment, is visible in the photographs in *A*.

All the abortive kernels shown in *A* and *B* were present on the right-hand cob in Plate 1*C*. Those in *A*, enlarged approximately ×6, are viewed from above. Those in *B*, enlarged approximately ×2½, are viewed from the side—the germinal side in the upper row and the abgerminal side in the lower row.

The abortive kernels at each end of the upper row in Plate 2*A*, and the 4 dark-pigmented abortive kernels on the left in 2*C*, have been included to illustrate another way in which the expression of a phenotype may be modified in a very special part of a tissue during its development. In the region of the silk attachment on the abgerminal side of the kernel, pigment is either absent, or present in low intensities, and basipetally directed forks extend from this region. For development of such patterns, pigment production must be inhibited in some cells during the later stages of ovary wall formation, or else previously formed pigment must be destroyed. All the cells derived from an initial segment of the basal ring may be subject to inhibition or destruction at practically the same stage in development, or the event may be initiated in only one or a few such cells, the effect spreading upward during subsequent growth to include adjacent cells.

The abortive kernels shown in Plate 2*C* were present on the tiller ear of plant 8597C-3. This ear had a large basal sector in which all kernels receiving a derivative of c_2^{m-2} had intense and uniformly distributed pigment in the aleurone layer. In Plate 2*D*, which shows a portion of the ear, a small segment of this basal sector is visible in two rows of kernels at the upper left. The sector contained 31 abortive kernels, and all had dark pigment in the pericarp layer. Seven of them are shown in Plate 2*C*. The white kernel on the left, with pigmented streaks on one side only, was located in a row adjacent to those that formed the sector of dark-pigmented kernels. The floral parts of the cob within the sector were not uniformly pigmented: subsectors displayed pigment intensities ranging from dark to nearly colorless.

Presetting of the Controlling Element at the Locus of c_2^{m-2}

The ear with the basal sector just described was instructive in other ways. An active *Spm* was present in the cells of that sector and in those of several rows on each side of it, but only in their basal part. The

c_2^{m-2} locus in the *Spm*-carrying cells of these adjacent rows had not been modified. The response of the locus to *Spm* resulted in pigmented spots in a colorless background in the aleurone layer of mature kernels. No active *Spm* was present, however, in the cells that produced the remainder and larger part of the ear, and no pigment appeared in the aleurone layer of any kernels within that part. Nevertheless, control of patterns of pigmentation was made evident by pigment distributions in the floral parts of the cob and in the pericarp layer of the mature kernels. In the middle of the photographed portion of the ear was a sector, spanning two rows, where the pericarp layer of the kernels was pigmented throughout, although the color intensity was greatly reduced in the region of the crown. Below the right-hand portion of this pigmented sector and extending to the right of it was another large sector, in which none of the kernels showed pigment in the pericarp layer. In kernels to the left of these two sectors, deep pigment appeared in the pericarp but only as sectors in individual kernels.

Pigment distributions similar to those illustrated in Plate 2*D* occurred on ears of other plants, where there was no evidence of the presence of *Spm* in the cells producing the ears. Such phenotypes appeared, however, only on ears of plants that had commenced development with an active *Spm*. This indicates that the controlling element at c_2^{m-2} responded to *Spm* early in plant development and was thus conditioned to control patterns of gene action in tissues produced very much later, even though an active *Spm* was no longer present. In other words, the initial response to *Spm* "preset" the element to undergo specific types of change—particular "settings"—often many cell generations after the presetting event had occurred. Each such setting then effected a special expression of the gene in the mature tissues subsequently formed by descendants of the cell in which it had occurred. Studies to be described in the next section suggest that many of the sectors observed on ears, in the floral parts of the cob as well as the pericarp layer of the kernels, arose through similar presetting and subsequent setting events, and that removal of an active *Spm* is not a requirement for the subsequent settings.

Inheritance of Modified Pigmentation Patterns

Studies were undertaken to determine the inheritance of some of the modified types of pigmentation patterns illustrated in the ears and cobs of Plates 1 and 2. Until very recently, material for such studies was limited. It was decided, therefore, to explore in a preliminary manner each available type in order to be able to select in the future those that would provide the most instructive information. The findings of these preliminary tests can be illustrated by describing the phenotypes of plants derived from kernels of the three ears whose cobs are shown in Plate 1*B*. As was mentioned earlier, all three of these ears were produced by cells that carried an active *Spm* element.

Two classes of kernels, present in equal numbers, were carried on the left-hand cob in the photograph. One had intense pigment throughout the aleurone layer; the other had none. The pigmented kernels received from the ear parent a derivative of the initial c_2^{m-2} locus that had been modified in the somatic cell whose descendants produced the ear. The colorless kernels were homozygous for the standard recessive c_2. Both types had dense pigment in the pericarp layer.

Plants derived from 13 of the pigmented kernels were intensely pigmented throughout. The floral parts of their cobs, unlike those of the parent cob, also were deeply pigmented, the color intensity resembling that of the dark sectors in the other two cobs in Plate 1*B*. These plants transmitted the modified c_2^{m-2} locus, now performing as a stable C_2 allele, in a strictly Mendelian manner. An active *Spm* was present in each of 11 plants tested for it, but the locus no longer responded to it.

Plants derived from 12 of the kernels having a colorless aleurone layer also were pigmented, but the color intensity was very much lower than in the sister plants derived from the colored kernels. An active *Spm* was present in each of 11 plants tested. Since these plants were homozygous for c_2, no somatically occurring change in gene expression was expected and none was noted.

Plants were grown from selected kernels on the middle cob in Plate 1*B*. The basal sector of this cob where the floral parts were deeply pigmented contained 6 kernels, in which the pericarp layer also was densely pigmented. This sector arose from the descendants of a cell in which the c_2^{m-2} locus had been modified to allow a C_2 type of expression in the floral parts of the cob and the pericarp layer of the kernels. The aleurone layer of 3 of these kernels had pigmented spots in a nonpigmented background—the expression given by an unaltered c_2^{m-2} locus in the presence of *Spm*. Obviously the modification of the locus that allowed C_2 expression in the sporophytic tissues did not effect a similar expression in the endosperm tissues. The aleurone layer of a fourth kernel was deeply and uniformly pigmented, indicating that a subsequent modification of the c_2^{m-2} locus had occurred before fertilization. The remaining 2 kernels had a colorless aleurone layer.

The plant derived from the kernel with a darkly pigmented aleurone layer was deeply pigmented in all its parts. The floral parts of the cobs were densely and uniformly pigmented, like the sector of the parent cob from which the kernel came. The pericarp layer of all kernels was darkly pigmented. Not only in the plant parts, but also in the aleurone layer of all kernels that received the modified c_2^{m-2} locus, the phenotype resembled that produced by the standard C_2 locus. An active *Spm* was present in the nuclei of this plant, but the modified c_2^{m-2} locus no longer responded to it in any detectable manner.

In plants derived from the 3 kernels with pigmented spots in a nonpigmented background, anthocyanin distributions and intensities resembled in range of types those of plants whose cobs and kernels are illustrated in the plates. In that regard, these 3 plants were indistinguishable from plants derived from 8 other kernels on the same parent ear that had pigmented spots in their aleurone layer. The modification of the c_2^{m-2} locus that was responsible for the deep-pigmented basal sector in the parent cob was not transmitted unaltered to the progeny.

The phenotype of the plants derived from the 2 kernels with a colorless aleurone layer resembled that of plants homozygous for the standard recessive c_2. *Spm* was present in each plant, but no somatically occurring modifications affecting anthocyanin distribution were noted. Also, the aleurone layer of all kernels on the testcross ears was colorless. These 2 plants probably were homozygous for c_2.

On the right-hand cob in Plate 1*B*, all kernels had a colorless aleurone layer, indicating that an early-occurring response to *Spm* of the element at the c_2^{m-2} locus had nullified its capacity to contribute to pigment production in this layer. Nevertheless there were several distinct sectors where the floral parts were deeply pigmented, and kernels located within these sectors had deep pigment in the pericarp layer. The appearance of such sectors indicated that the element at c_2^{m-2} had undergone subsequent modifications during development of the ear. These changes allowed intense pigment to be produced in sporophytic cells but not in the aleurone layer of the kernel. Because the aleurone layer was alike in all kernels, those that received a modified c_2^{m-2} locus could not be distinguished from those that received the standard recessive c_2.

Plants were grown, therefore, from the 5 kernels located within the dark-pigmented sector of the cob that is visible in the photograph, and from 12 other kernels located elsewhere on the cob (not in dark-pigmented sectors). The 17 plants were pigmented, but the intensity of pigmentation resembled that in plants homozy-

gous for c_2. None of them was variegated for different intensities of pigmentation within a tissue, as the ear parent had been, even though *Spm* was present in 12 of the 13 plants tested for it. Also, the aleurone layer of all kernels on ears of the plants, produced by the testcross, was completely colorless. None of the cobs, including those of plants derived from kernels from the dark-pigmented sector of the parent cob, exhibited deep pigmentation in the floral parts. Very probably some of the 12 plants known to have had an active *Spm* element received from the ear parent the modified derivative of c_2^{m-2}. Further tests will be required to determine whether such plants may be distinguished from those that are homozygous for the standard recessive. Present evidence suggests, however, that the c_2^{m-2} locus lost its capacity to undergo further modifications that would allow it to contribute to formation of intense pigment in the sporophytic tissues.

Other tests similar to those just described were conducted. In addition, individual kernels were selected from some ears and the plants derived from them were examined for transmission of the kernel phenotypes. Some of the selected kernels had a fully pigmented aleurone layer. Others came from ears having sectors in which the aleurone layer was colorless but the pericarp layer was either colorless or variegated, or fully pigmented. Still others were selected from ears of plants that commenced development without an active *Spm* element. None of these last-mentioned plants showed evidence of any somatically occurring change in control of c_2^{m-2} gene action, nor did their progeny unless *Spm* had been introduced in the cross of the ear, from which kernels receiving *Spm* were selected. The plants derived from such kernels did show somatically occurring changes, whereas the phenotype of sister plants derived from kernels that did not receive *Spm* resembled that of the ear parent.

Results of tests so far conducted with plants carrying *Spm* and either c_2^{m-1} or c_2^{m-2} indicate that the modifications of these loci which occur during plant development and alter gene expression in the aleurone layer of the kernel are heritable in a Mendelian manner, whereas most changes affecting gene expression in the sporophytic tissues are not heritable in the same way. The modifications affecting sporophytic tissues can occur after *Spm* has been removed or inactivated, and bring about the same range of alteration in phenotypic expression as appears when *Spm* remains active.

This series of tests has led to the hypothesis of "presetting" and subsequent "setting" of the gene locus during development of the sporophytic tissues, mentioned earlier in this report. The nonheritability of the settings suggests that they are erased or modified, either in the germ line or in the zygote or developing embryo. There is some evidence, still incomplete, to suggest that when a modification occurs (instead of an erasure) it is the same in all cells whose c_2^{m-2} locus has undergone the same presetting and setting process, and that its effect is expressed either in the presence or in the absence of *Spm*.

Presetting and subsequent setting of a locus, to control the action of its gene many cell generations after the presetting event has occurred, may be a general attribute of control systems in maize. First evidence of the process, obtained with two states of a_1^{m-2}, was reported and illustrated in *Year Book 63* and *Year Book 64*. In that material, contrary to the findings reported here for c_2^{m-1} and c_2^{m-2}, the effect of the presetting and setting process is recorded in the aleurone layer of the progeny kernels of plants carrying *Spm*, but made evident only in those kernels that do not receive *Spm* from either the ear or the pollen parent. These settings are nearly always erased in the next plant generation at some period during development, possibly in the germ line. In a few cells, however, the locus may escape the erasure process.

There is now a considerable body of evidence that relates controlling elements to the ordering of gene action during

development. A single system, such as the *Spm* system, apparently accomplishes this function in a number of distinctly different ways. Each must reflect the nature of the elements themselves, their modes of association with the gene loci, and their individual actions and interactions. The relation of the elements to the gene loci has been investigated by Dr. O. E. Nelson of Purdue University. In studies (unpublished) of wx^{m-1}, wx^{m-6}, and wx^{m-8}, each of which arose through the insertion of a controlling element at the *Wx* locus, he has obtained evidence that makes it possible to determine the position of the element within the locus. The site of insertion is different in each of the three instances. The inserted element does not noticeably reduce intralocus crossing over. It is not yet possible, however, to relate controlling elements to other known structures, as our knowledge of chromosomal components is still too limited. Nevertheless, the precision with which the elements are able to regulate gene action reflects an orderly control mechanism, operating from the time of zygote formation until the time of maturation of tissues.

In eukaryotic organisms (plants and animals having complex chromosomes and well organized nuclei), the organization of the chromatin is not the same in all nuclei of an individual. Characteristic differences are observed in degree of condensation of chromatin within nuclei performing different functions. In some types of cells, much of the chromatin within a nucleus forms densely meshed clusters, often connected with the nuclear membrane. Biochemical studies have indicated that the degree of activity of genes within clusters is low by comparison with that of genes in chromatin loosely dispersed in the nucleus. Thus some mechanism must operate during development to order the genes into clusters, and to effect dissociation of particular genes from such clusters so that they may function at the proper times and to the proper degrees. It is possible to consider that in the regulation of this mechanism the controlling elements may be involved. Their initial positions and associations within a cluster might result in some modification that would alter their positions within the nuclei in subsequent cell generations. Each altered association, in turn, could affect the position of an element in one or more succeeding cell generations. Many of the observed complexities of action of the controlling elements in maize could stem from such a relatively simple sequence of events. The "presettings" and "erasures" reported here would appear less strange under this interpretation.

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